[D]epartment of Agriculture Agricultural Research Service July 1989

Agricultural Research

Where Will Tomorrow's Scientists Come From?

One of America's most basic resources is in peril. I'm referring not to soil, water, or air—although the threats to these natural resources are very real—but rather to our national base of scientific knowledge. The growing sophistication and complexity of today's problems cry out for scientific and technological solutions.

But where will tomorrow's scientists come from? As a nation, we seem almost to be turning our backs on science. Consider these sobering statistics. Out of every 1,000 graduates of U.S. high schools, only about 50 will go on to earn undergraduate degrees in science and engineering; fewer than 3 will earn doctorates in those disciplines. At this rate, the Nation will produce some 200,000 baccalaureate scientists and engineers and about 10,000 Ph.D's annually.

This isn't enough. Not when continued advances in agriculture, medicine and other sciences depend on a steady influx of talented young people with fresh ideas and perspectives. Not when graduation rates in science and engineering are much higher in other countries, including many of our trade competitors.

Compounding the problem is this fact: By the year 2000, more than half of the Nation's work force will be composed of women and members of minority groups—people who have traditionally been underrepresented in science and engineering. Women make up about 45 percent of the Nation's work force, but in 1986 earned only 23 percent of the Ph.D's in science and engineering. Blacks, 12 percent of the population, earned only 1 percent of the Ph.D's in those fields. Hispanics are 9 percent of the population, but earned only 2 percent of those Ph.D's.

We can attribute this dearth of Ph.D's earned by women and minorities to, perhaps, lack of opportunity, lack of interest, or lack of encouragement, but certainly not to lack of ability. Female and minority scientists have made, and are making, lasting contributions to society. This issue of *Agricultural Research*, in fact, highlights the accomplishments of several female and minority scientists in the Agricultural Research Service.

The point is that women and minorities are a too-often-overlooked source of scientists and engineers—a source that the Nation must draw upon more heavily if we hope to maintain our lead role in scientific and technological development.

I have recently been privileged to be a member of a White House Task Force on Women, Minorities, and the Handicapped in Science and Technology. Our job was to help develop recommendations for increasing the size and diversity of our graduating classes in science and engineering, particularly among these underrepresented groups.

Our report called for the federal government, universities, local school boards, and private enterprise to create a climate in which science can thrive. But there is also room—and in my mind an imperative—for individuals to make a significant contribution.

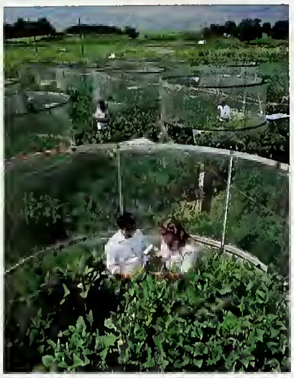
I believe that everyone involved in science has a personal responsibility to help shape the local educational agenda. We all need to become active supporters of science education, and if we do, our combined efforts can make a difference! We cannot allow our schools to postpone studies in basic science and mathematics even until junior high, or we can count on losing many of our best and brightest students to other areas of study.

Specifically, we can:

- Work with local school boards to strengthen programs in science and mathematics.
- Volunteer to help teachers develop and teach science curriculums, and to help out with special projects, such as annual science fairs.
- Interest the local media in our work so we can let the people in our communities know how their lives are enriched by what we do.
- When discussing science with nonscientists, remember to drop the lab jargon and use terms that they can understand.
- Participate in adopted, or mentor, school programs that invite students to the lab for visits, special projects, or even summer jobs. This concept has proven successful at several schools across the Nation, such as Chicago's High School for the Agricultural Sciences, Philadelphia's Walter Biddle High School, and Washington, D.C.'s, Van Ness Elementary School.
- Make students aware of the opportunities for careers in science and engineering—careers that are satisfying and rewarding, and that will be the foundation for our country's future.

Mary E. Carter
Associate Administrator
Agricultural Research Service

Agricultural Research



Cover: In open-top chambers at Beltsville, Maryland, ARS researchers in cooperation with the University of Maryland study the effects of air pollution on soybean plants. Story on page 4. Photo by Keith Weller.

Page 14

Page 16

Page 10

DEPARTMENTS

Vol. 37, No. 7
July 1989

Special Editor: Sandy Miller Hays
Photography: Robert Bjork, Anita Daniels, John Kucharski

Agricultural Research is published by the Agricultural Research Service (ARS), U.S. Department of Agriculture, Washington, DC 20250. The Secretary of Agriculture has determined that publication of this periodical is necessary in the transaction of the public business required by law of the Department.

Information in this magazine is public property and may be reprinted without permission. Prints of photos (except those copyrighted) are available to mass media; please order by photo number and date of magazine issue.

Subscriptions: $14.00 for 1 year in the United States or $17.50 foreign. Send orders to Superintendent of Documents, Government Printing Office, Washington, DC 20402. Request *Agricultural Research,* stock number 701 006 00000 3.

Magazine inquiries or comments should be addressed to: The Editor, Information Staff, Room 316, Bldg. 005, Beltsville, MD 20705. Telephone: (301) 344-3280. When writing to request address changes or deletions, please include a recent address label.

Clayton Yeutter, Secretary
U.S. Department of Agriculture

Charles E. Hess, Assistant Secretary
Science and Education

R.D. Plowman, Administrator
Agricultural Research Service

Wanted: Breathing Room for Crops

KEITH WELLER

Plant physiologists Edward Lee (right) and Randy Rowland analyze data of plant tissue exposed to ozone pollution and CO_2 enrichment. (K3205-9)

SCIENTISTS with the Agricultural Research Service are gearing up for what could be one of American agriculture's toughest struggles in the coming century: the battle to breathe.

American farmers already lose to air pollution an estimated $3 billion a year in damaged crops, according to Edward H. Lee, an ARS plant physiologist. Lee works at the agency's Climate Stress Laboratory at Beltsville, Maryland.

Lee says cutting just one of the pollutants, ozone, by 10 percent would save farmers $808 million in 1982 dollars, while a 25-percent reduction in ozone pollution could boost farmers' income $1.89 billion annually and a 40-percent reduction would mean an extra $2.78 billion for agricultural producers.

Science has already scored at least one victory. Ethylenediurea (EDU), a plant growth regulator, has been found to help plants fend off the assaults of air pollution, says Lee.

"We've been able to treat plants with EDU and cut damage," he notes. "If you treat red clover with 50 milligrams of EDU—500 parts per million—in a 5-inch pot for 48 hours before you subject it to high ozone stress, you can change a very sensitive plant to an insensitive one.

"Under laboratory conditions, a single soil drench was effective in providing protection against acute ozone exposure. But the efficacy of these compounds for field application must still be established."

Scientists reported in early 1986 that injections of EDU in the stem of shade trees could protect the trees from ozone damage. Test results at that time indicated that EDU altered enzyme and membrane activity within the leaf cells where photosynthesis takes place.

In addition to ozone, other major air pollutants damaging to crops include peroxyactetyl nitrate, oxides of nitrogen, sulfur dioxide, fluorides, agricultural chemicals, ethylene, and other hydrocarbons, Lee says.

"But almost 90 percent of the crops lost to pollutants are lost because of ozone and sulfur dioxide," he adds. "And ozone is about 10 times more toxic than sulfur dioxide. Ozone affects vegetation throughout the United States, impairing crops, native vegetation, and ecosystems more than any other air pollutant."

While stratospheric ozone has been much in the public eye in the past year, with its connection to the "greenhouse effect" and Earth's weather, it is the atmospheric ozone closer to Earth that wreaks havoc on crops, Lee says.

When ozone's effect on crop plants is visible, it takes the form of chlorosis, where the plant turns yellow, or necrosis, where spots on the plant's cells have died. Yellowing occurs because ozone destroys chloroplasts that make chlorophyll, which gives plants their green color. Chlorophyll transforms light energy from the sun into chemical energy for the making of plant food from carbon dioxide and water—the process called photosynthesis.

In addition to reducing photosynthesis, ozone's unseen effects on crop plants can include weakened cell walls that allow important nutrients to leach out, as well as more rapid aging of the plant.

Ozone damage also opens the door wider to another threat: pests.

"A stressed leaf has a higher sugar content," Lee explains. "Insects can sense that sugar, and they like sugars. In an experiment where Mexican bean beetles were given a choice of snapbean leaves that had suffered stress from sulfur dioxide or ozone, or a plant that had been grown in filtered air, they went directly to the stressed leaves. So pollutants increase the insect population and double the damage."

Fortunately for farmers, some plants are better able to tolerate high ozone concentrations, and differences exist among varieties within a species such as wheat.

KEITH WELLER

In cooperative studies at the Beltsville Agricultural Research Center, University of Maryland agronomist Charles Mulchi observes the effects of the chemical protectant ethylene-diurea (EDU) on tobacco leaves. (K3204-9)

Among the more hardy plants, those able to stand exposure to 0.35 parts per million of ozone for 1 to 2 hours without visible damage, are zinnias, radishes, poinsettias, black walnuts, strawberries, and carrots.

Somewhat less tolerant—starting to show damage after 1 to 2 hours at 0.2 parts per million—are begonias, onions, chrysanthemums, dogwood, sweet corn, wheat, and lima beans. The most sensitive species, showing damage after a couple of hours'exposure to 0.1 parts per million, include spinach, musk-melons, oats, pinto beans, white pine, potatoes, and tomatoes.

"We're studying how plant hormones such as gibberellins may be involved in why a plant is sensitive or insensitive to pollutants," says Lee. "Also, enzymes in the plant tissue can help fight toxicity. We're trying to find out if sensitive plants have less detoxification enzymes."

The research agency has been studying ozone's impact on plants since 1962, including work in the laboratory and greenhouse as well as field work at Beltsville and Richmond, Virginia. The University of Maryland has been cooperating with the agency on the studies since 1983.

In studies at Beltsville in 1980, scientists determined that increasing ozone levels from 0.05 parts per million to 0.10 parts per million could slash soybean and corn production by 20 percent. Comparisons of crop output with ozone levels raised to 0.10 parts per million from 0.02 parts per million for 7 hours showed losses of 37 percent for winter wheat, 18 percent for rice, 12 percent for summer wheat, and 10 percent for sorghum, Lee says.

Comparing production in nonfiltered air at Beltsville with crops grown in filtered air, three varieties of potatoes averaged 10 percent more output in the filtered air, four varieties of sweet corn averaged a 9-percent gain, four varieties of snapbeans averaged 4 percent more yield, one type of tomatoes showed a 17-percent improvement, and two varieties of soybeans averaged a 10-percent gain.

KEITH WELLER

Biological aide Margaret Landis uses a porometer to measure the impact of CO_2 enrichment on the transpiration rate and stomata activity of a soybean leaf. (K3204-1)

But all beans are not created equal when it comes to ozone tolerance. As an example, Lee points to the snapbean variety BBL-290, known to be particularly susceptible to ozone pollution.

When left to fend for itself in unfiltered air, BBL-290 produced only 86 percent as much as when it was coddled in carbon-filtered air. But its cousin Astro, a more ozone-tolerant variety, produced 97 percent as much in the nonfiltered air as it yielded in a cleaner atmosphere.

Ozone levels rise steadily through the morning to peak around noon, declining rapidly in the afternoon, says Lee.

A soybean leaf from a plant inoculated with rhizobium (on left) shows greater tolerance to ozone stress compared with a discolored leaf (on right) from a plant that was not inoculated with rhizobium. (K3204-15)

"It's a photochemical compound," he explains. "The compound nitrogen oxide is required; it comes from automobile exhaust. Light acts on it and turns it to ozone."

"Clean" air might have an ozone level of 0.01 to 0.02 parts per million, while the air in an industrial city on a smoggy summer day might contain 0.1 to 0.2 parts per million of ozone, and ozone levels in an urban atmosphere might peak at 0.5 to 0.8 parts per million.

A recent check of ozone levels around the world showed readings of 0.145 parts per million for Bonn, West Germany; 0.147 parts per million for London, England; 0.274 parts per million for Los Angeles, California; 0.160 parts per million for Osaka, Japan; 0.372 parts per million for Riverside, California; 0.190 parts per million for Tokyo, Japan; and 0.156 parts per million for Washington, D.C.

Studies under way in cooperation with the University of Maryland (UM) are focusing on the effect of high concentrations of carbon dioxide and ozone and enhanced levels of ultraviolet radiation on the world's crops, Lee says. Charles L. Mulchi, an associate professor of agronomy at UM, is working with Lee and plant physiologist Donald T. Krizek of ARS' Climate Stress Laboratory.

"Global carbon dioxide is increasing about 1 percent a year because of the burning of fossil fuels," says Mulchi. "It's now about 350 parts per million, and we're projecting that 30 years from now, it will be about 450 parts per million."

Lee, Krizek, and Mulchi are experimenting with growing corn, soybeans, and wheat in special growth chambers in which they can control the levels of carbon dioxide, ozone, and UV-B, the type of ultraviolet radiation associated with tanning and skin cancer. The growth chambers are set in the fields of ARS' South Farm at Beltsville.

"Ozone in the stratosphere serves as a filter to take out UV-B," says Mulchi. "But fluorocarbons and some hydrocarbons get into the stratosphere, cause ozone depletion and let in more UV-B. This also causes more ambient ozone closer to Earth because that ozone is produced by light.

"Our study is to look at all three variables—an increase in carbon dioxide, high levels of pollution, and several levels of UV-B. We'll be doing this for a minimum of 4 years.

"High carbon dioxide causes plants to grow faster, and it thickens up their leaves," Mulchi continues. "But will that make them less vulnerable to the effects of UV-B? We don't know yet."

Increased UV-B levels have been found to have ill effects on crop yield and quality in nearly half of the species examined to date, says Lee.

"Still, there are many cultivars of agricultural crops that show resistance to increased UV-B," he says. "Studies are needed to find out why this is true, under environmental conditions that are well defined."

"Whatever we find will help us get an idea of how major crops will grow under these conditions," adds Mulchi. "Another part of the study will look at the internal mechanism of plants to adapt to these changes.

"If we can get a handle on what controls the changes in plants under these conditions, we could go to plant breeders and say, "This is the genetic material, find it and breed it into the plants for the 21st century."

Mulchi said the study will focus not only on the quantity of crops grown under atmospheric conditions that may develop in the next century, but also on the quality of those crops.

"It's our opinion you really need to look at all the variables in concert to make policy decisions for the future, like should we go more to nuclear energy or should we use less gas in our cars," he concludes.—By **Sandy Miller Hays**, ARS.

Edward H. Lee and Donald T. Krizek are at the Climate Stress Laboratory, Beltsville, MD 20705 (301) 344-3143. Charles L. Mulchi is at the University of Maryland Department of Agronomy, College Park, MD 20784 (301) 454-3717. ◆

"Treasure Maps" to Pest Control

You can't find the treasure without the map.

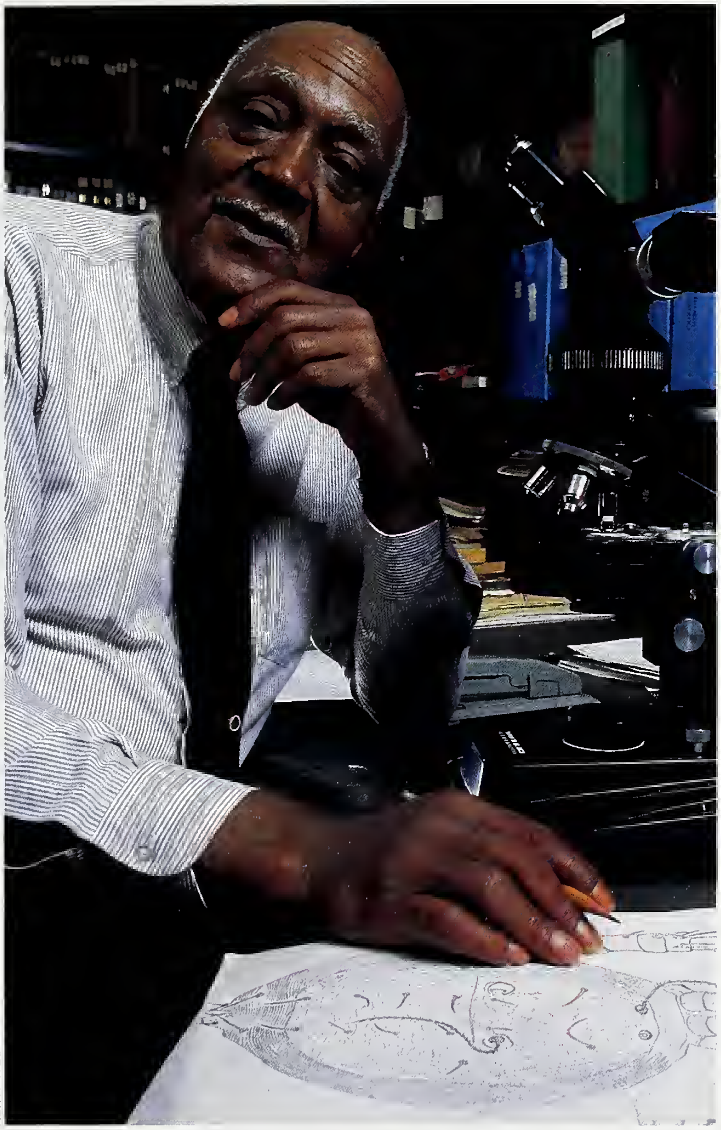
As the Agricultural Research Service searches for natural pest controls, more than a few of its crucial "treasure maps" come from the drawing board of research entomologist Robert L. Smiley.

Smiley works at ARS' Systematic Entomology Lab in Beltsville, Maryland, where he is concentrating his efforts on the cunaxid mite. His work in outlining each spine, spur, and claw of the cunaxid will help researchers in development of biocontrols against pests.

The process of mapping the mites is called "taxonomy" — the orderly classification of plants and animals.

"It ensures that you're getting the right species," Smiley says. "This information can be used by ARS and other research agencies to help replace pesticides."

The cunaxid is found in the bark of citrus and fruit trees as well as in stored food products. The mite also takes up residence in woody plants, humus, leaves, moss, and straw.



KEITH WELLER

Entomologist Robert L. Smiley has made highly detailed drawings of 185 species of the cunaxid family of mites from specimens sent to him from researchers around the world. His drawings will be published in an encyclopedia on the mite for use by biocontrol experts. (K3210-1)

Smiley's work with the cunaxid mite could prove to be a key factor in fulfilling ARS' mission of finding biocontrols for pests in citrus crops, grapes, and stored products. The cunaxid is a natural predator of certain other mites, scale insects, and arthropods that damage these crops and products.

For example, the eriophyid gall mite, *Calepitrimerus vitis*, damages grape vines. But *Cunaxoides oliveri*, a type of cunaxid, has been reported in Europe as a predator of the gall mite. Similarly, *Cunaxoides parvus* is an enemy of the oyster shell scale, an insect that attacks a variety of trees and shrubs.

"What we're looking for is the correct classification of these mites," says Smiley. "It's one step, but there are a lot of ramifications for biological control."

Smiley began his detailed drawings of members of the cunaxid family on a limited basis in 1975 because only a general description of the cunaxid mite existed. Emphasis on the research was stepped up in 1986 in conjunction with ARS' increasing attention to biocontrol.

Since Smiley started his work on the cunaxid, he has reviewed approximately 6,000 specimens and produced detailed drawings of 185 species of the mite.

ARS will publish Smiley's encyclopedia of the cunaxid family for Federal and State agencies interested in using the mite as a biocontrol. Smiley hopes to have a first draft ready by this fall.

Although the cunaxid is found world-wide, Smiley has not personally had the opportunity to look for various species. Instead, scientists from such farflung spots as Australia and Thailand have sent species and information about their prey to the Beltsville lab.

Smiley says none of the species he has outlined are currently being used in biocontrol programs.

But another family—the phytoseiids—has a proven track record as a biocontrol against mites on crops and stored products. One phytoseiid, *Typhlodromus occidentalis*, cut pesticide costs by $5 million annually for mite control on apples grown in the State of Washington.

Smiley is hoping his work with cunaxids will help the mite mimic the success of the phytoseiids, and help reduce the opportunities for chemical contamination of soil and water.—By **Bruce Kinzel**, ARS.

Robert L. Smiley is at the USDA-ARS Systematic Entomology Laboratory, Beltsville, MD 20705 (301) 344-3891. ◆

Fungal Troublemaker Under Fire

FROM scabby grain to moldy corn toxicosis, *Fusarium* fungi are known troublemakers when it comes to plant disease and, indirectly, animal health.

Scientists like organic chemist Odette L. Shotwell have known for years that *Fusaria* living on plants produce toxic chemicals. Shotwell leads the Agricultural Research Service Mycotoxin Research Unit at the Northern Regional Research Center in Peoria, Illinois.

But the details of how these fungi live, invade plant tissue, and form toxins are still being studied. Investigating these details are four scientists with diverse backgrounds: Marian N. Beremand, molecular geneticist; Anne E. Desjardins, biochemist; Thomas M. Hohn, microbiologist; and natural products chemist Susan P. McCormick.

These four, along with chemist Ronald D. Plattner, are working toward a goal of protecting crops and stored grains from contaminating toxins.

To better understand the mechanisms by which fungal infections establish themselves and survive, research has focused on how and why *Fusarium* molds make certain types of poisons called tricothecene toxins, on how the plant makes natural defense chemicals called phytoalexins, and on the chemical interactions between the plant and the troublesome fungus.

Understanding the steps a fungus uses to make trichothecenes is critical to developing methods to prevent toxin production. Once the steps are known, the scientists will design experiments to perhaps manufacture specific inhibitors that block the pathway.

Beremand began her studies by exposing one strain of *Fusarium* to ultraviolet light. She selected this strain because it attacks corn. Exposed to the radiation, the single parent strain produced 10,000 strains. Among these, Beremand found mutant strains that would make different kinds or amounts of trichothecenes.

Using three of the mutant strains, McCormick isolated and purified a number of possible precursors of a trichothecene, T-2 toxin, including nine new natural products. Beremand, McCormick, and their coworkers used the compounds to determine the sequence of all but 4 of the 13 or 14 steps used by the fungus to make T-2 toxin.

Each step in the biological pathway is controlled by one or more genes, but until now scientists didn't have the tools to study these genes. Beremand and Desjardins developed the first and only genetic system to study trichothecene production in *Fusarium.* With this system, scientists may now identify and characterize the genes regulating formation of T-2 toxin.

The first step was taken when Hohn identified and characterized the first enzyme in the pathway to make trichothecenes. He also isolated a gene from *Fusarium* that produced T-2 toxin and from this he produced a gene clone.

Desjardins and several coworkers were the first to discover that trichothecenes are necessary for *Fusarium* to cause plant disease.

"These results suggest that compounds that block the fungus from making trichothecenes would also prevent the fungus from infecting plants," Desjardins says.

In nature, there's an ongoing struggle between the plant and the fungus. While trichothecenes are necessary for the fungus to infect the plant, the plant fights back by making phytoalexins that are toxic to the fungus.

The fungus responds by producing enzymes that inactivate these phytoalexins. Desjardins and coworkers discov-

BRUCE FRITZ

Research group for *Fusarium* mycotoxin biosynthesis in their laboratory at the Northern Regional Research Center, Peoria, Illinois. Clockwise from front are organic chemist Odette Shotwell, research associate Yangkyo Salch, chemist Susan McCormick, microbiologist Thomas Hohn, chemist Harold Gardner, biochemist Anne Desjardins, and geneticist Marian Beremand. (K3201-8)

ered that disease-causing *Fusarium* strains can break down phytoalexins from parsnips and potatoes into compounds that are not toxic.

"Ideally, if the plant's own defense mechanism could be altered or helped to inhibit the fungus, there could be less reliance on chemical fungicides," says Desjardins.

One alternative to fungicides is a genetic approach, recently developed by Beremand and Yangkyo P. Salch. The scientists are inserting various genes into the fungus, hoping to make the fungus harmless to the plant.

Although the questions that Beremand, Desjardins, Hohn, and McCormick are investigating are primarily of agricultural interest, there is another aspect as well.

Their studies run parallel to one of the first major achievements of the Peoria lab. In the early 1940's, studies at the lab on a strain of *Penicillium* were instrumental in helping launch the antibiotics industry.

Now Beremand's genetic experiments with trichothecenes coincide with renewed interest by medical researchers in trichothecenes as potential anticancer compounds.

"Trichothecenes are interesting because when they are attached to compounds that bind only to cancer cells, the toxic effect of the trichothecene kills only cancer cells while leaving healthy cells alone," Beremand says.

"If such compounds prove to be medically useful, researchers will need a large amount and variety of tricothecenes with which to experiment.

"From the same mutants used to identify the genetic system, we have identified 10 new strains that make ideal candidates that could supply compounds for medical research." —By **Linda Cooke**, ARS.

Marian Beremand, Anne Desjardins, Thomas Hohn, Susan McCormick, and Odette Shotwell are at the USDA-ARS Northern Regional Research Center, 1815 N. University St., Peoria, IL 61604 (309) 685-4011. ◆

Treatment Lands Fruit Pests in Hot Water

BOB BJORK

Printout from a computer-controlled test of hot-air treatment to kill insect eggs and larvae in shrink-wrapped grapefruit is studied by entomologist Jennifer Sharp. Technician Rick Duncan operates the computer. (K3183-10)

Haiti, Mexico, Jamaica, Costa Rica, Guatemala, Brazil, Venezuela, and Peru—for most people, these words may bring to mind sandy beaches, warm breezes, tall cool drinks, and gently swaying palms.

But that's not the vision that Jennifer L. Sharp imagines when these exotic places are mentioned. Instead of fun in the sun, she thinks of flies and the fruit they attack—mangoes, grapefruit, avocados, and papayas, infested with fruit fly eggs and larvae.

This type of thinking is quite natural for Sharp, an Agricultural Research Service entomologist, since she is research leader at the agency's Subtropical Horticulture Research Laboratory in Miami, Florida. The station serves as the U.S. port of entry for new crops, and is home to national germplasm collections for tropical and subtropical crops.

"Part of our mission is to find and develop effective quarantine treatments for fruit flies," Sharp says. "In this role, we work closely with the Animal and Plant Health Inspection Service."

According to Chuck Havens of APHIS' Port Operations in Hyattsville, Maryland, exotic fruit imported into the United States from a country where exotic fruit flies exist cannot bear any live quarantine insects.

This means a treatment is needed to make the product 100 percent free of fruit flies from the country of origin.

In 1987, Sharp developed a hot-water immersion treatment that destroyed 100 percent of fruit fly larvae in mangoes from Haiti.

Since then, she has been a technical adviser and project leader in some instances on a similar program for Mexico, Jamaica, Costa Rica, Brazil, Guatemala, Venezuela, and Peru.

Sharp's hot-water immersion tank is easily assembled, durable, and mobile. After 75 minutes in the 115.5-degree-Fahrenheit bath, Francis mangoes emerge free of fruit fly larvae and show no adverse effects.

"Other countries can also benefit from this research," says Milton T. Ouye, ARS national program leader for product losses and quarantine.

"Based on current mango data, we've sent a recommendation to APHIS to allow this technology to be used in Puerto Rico, southern Mexico, the West Indies, and Central America, except Panama."

For 27 Caribbean Basin countries, the 1983 Caribbean Basin Economic Recovery Act spelled valuable duty-free access to U.S. markets.

But the United States won't accept horticultural imports that are infested with fruit fly larvae or that have been treated with ethylene dibromide (EDB).

"These countries need help in finding an alternative since the Environmental Protection Agency banned the use of EDB on mangoes in 1987 and on all other fruits in 1984," Sharp says.

Larvae of the Caribbean fruit fly, *Anastrepha suspensa*, in Florida mangoes have succumbed to Sharp's hot-water treatment. In addition, both the Caribbean species and the West Indian species, *Anastrepha obliqua*, have capitulated in Haitian mangoes.

ROBERT BARCLAY

BARRY FITZGERALD

**Left: Caribbean fruit fly laying an egg in a grapefruit. (K898-8)
Right: Just beneath a grapefruit peel, fruit fly larvae begin to devour the pulp. Hot-air treatment effectively kills the costly pest. (K1674-7)**

Sharp also has successfully treated Mexican mangoes for eradication of the West Indian species of fruit fly, the Mexican fruit fly, *Anastrepha ludens*, and the Mediterranean fruit fly.

According to the Foreign Agricultural Service, the United States last year imported 34,646 metric tons of mangoes valued at $28.9 million, principally from Mexico.

At the ARS Tropical Fruit and Vegetable Research Lab in Hilo, Hawaii, John W. Armstrong developed another technique for papayas.

Armstrong gradually heated papayas for about 7 hours with hot air at 40- to 60-percent relative humidity until the temperature at the fruit's center reached 117 degrees Fahrenheit.

Not only does this treatment kill all stages of the flies as required by Government regulations, but it also kills eggs and larvae hidden deep in the papayas without destroying the fruit—something hot-water immersion can't accomplish. Armstrong says his treatment can be used for other agricultural commodities as well.

Building on Armstrong's research, Sharp is using the hot-air technique on grapefruit and other citrus, avocados, mangoes, and carambolas.

Tests on grapefruit showed that a 3-hour hot-air treatment at approximately 115 degrees Fahrenheit effectively killed mature Caribbean fruit fly larvae.

Carambolas subjected to temperatures of approximately 115 degrees Fahrenheit for 2 hours were undamaged and free of pests.

Sharp extended the length of treatment on grapefruit to 8 hours, again at 115 degrees Fahrenheit. The grapefruit was then stored for 4 weeks at approximately 59 degrees Fahrenheit.

When compared with untreated fruit, the treated grapefruit showed no noticeable differences in firmness, color, sugar content, or aroma. In subsequent informal taste tests, more people indicated that they preferred the treated fruit.

"We're now treating grapefruit with hot air at about 126 degrees Fahrenheit for 4 hours," says Sharp. "We're also looking for any change in fruit quality."
—By **Doris Sanchez**, ARS.

Jennifer L. Sharp is research leader at the USDA-ARS Subtropical Horticulture Research Station, 13601 Old Cutler Road, Miami, FL 33158 (305) 238-9321. ◆

Nitrogen Overload May Shrivel Vitamin Content

EXCESSIVE nitrogen fertilizer can slash vitamin C content in some leafy greens by up to half, soil scientist Sharon B. Hornick has found.

Hornick made the discovery in a series of experiments that began when she tried to shed some light on the old question of whether food's nutritional quality is better with organic rather than inorganic fertilizers.

"It turned out that too much nitrogen was bad regardless of whether it was synthetic or organic," says Hornick, who works at the ARS Soil-Microbial Systems Laboratory at Beltsville, Maryland.

"It's important to remember that this research is at an early stage," Hornick says. "Large-scale field tests will be needed before the results can be extrapolated to what may occur in commercial horticultural production."

Hornick grew swiss chard and green beans in potted soil in a greenhouse. Some of the plants received 3-1-1 manure—manure with 3 percent nitrogen, 1 percent phosphorus, and 1 percent potassium.

Other plants received 2-1-1 sewage sludge compost. The fertilizer was applied at rates equivalent to zero, 9, 18, 27, and 36 tons per acre.

For comparison, those plants receiving no manure or compost did receive inorganic fertilizer at rates then recommended for commercial vegetable growers in Maryland. Those rates equalled 60 pounds of nitrogen, 36 pounds of phosphorus, and 24 pounds of potassium per acre for beans, and 75 pounds of nitrogen and 100 pounds each of phosphorus and potassium for the chard.

To Hornick's surprise, the vitamin C content decreased as she added more compost or manure.

To determine the vitamin C content, bean leaves were harvested

after 30 days and analyzed immediately. The same analysis was done for chard, harvested after 35 days.

As an example of the decline in vitamin C, chard leaves grown without compost had 81.4 milligrams of vitamin C per 100 grams of leaves. At the highest compost rate, vitamin C content was only 54 milligrams per 100 grams of leaves.

Next, Hornick eliminated the organic fertilizers and separately tested inorganic nitrogen, phosphorus, and potassium.

"In my second experiment, it was clear that high nitrogen rates were responsible for the lowering of vitamin C levels seen in earlier experiments," she says.

Hornick then tested various amounts of inorganic nitrogen, using rates equivalent to 100-500 pounds per acre for farmers. She also switched to kale, a plant high in both vitamin C and iron.

Again, as the nitrogen went up, the vitamin C went down, as much as 50 percent at the highest level.

Hornick eliminated the soil as a contributing factor by growing the kale hydroponically, without soil. She also changed the nitrogen rates to the equivalent of 10-100 pounds per acre, amounts commonly used by farmers. Once again, the impact on vitamin C levels was dramatic.

"There was no significant difference in the yields of chard and beans grown with different rates of compost," Hornick notes.

"This, coupled with the variability of yields at the higher nitrogen rates, makes it unlikely that the sole explanation is that the plants diluted the vitamin C by growing faster and bigger."

Kale is an important potential food source worldwide because it is high in both vitamin C and iron. Vitamin C aids in human absorption of iron, an element that is underrepresented in many diets around the world.

Hornick plans to eventually feed kale grown in soil treated with various levels of nitrogen to people or animals to see whether the level of iron in their blood changes.

Meanwhile, Hornick will test spinach and other leafy vegetables to see if high vitamin C content increases the amount of available iron in food. She will do this by simulating human digestion with stomach and intestinal enzymes in a test tube.

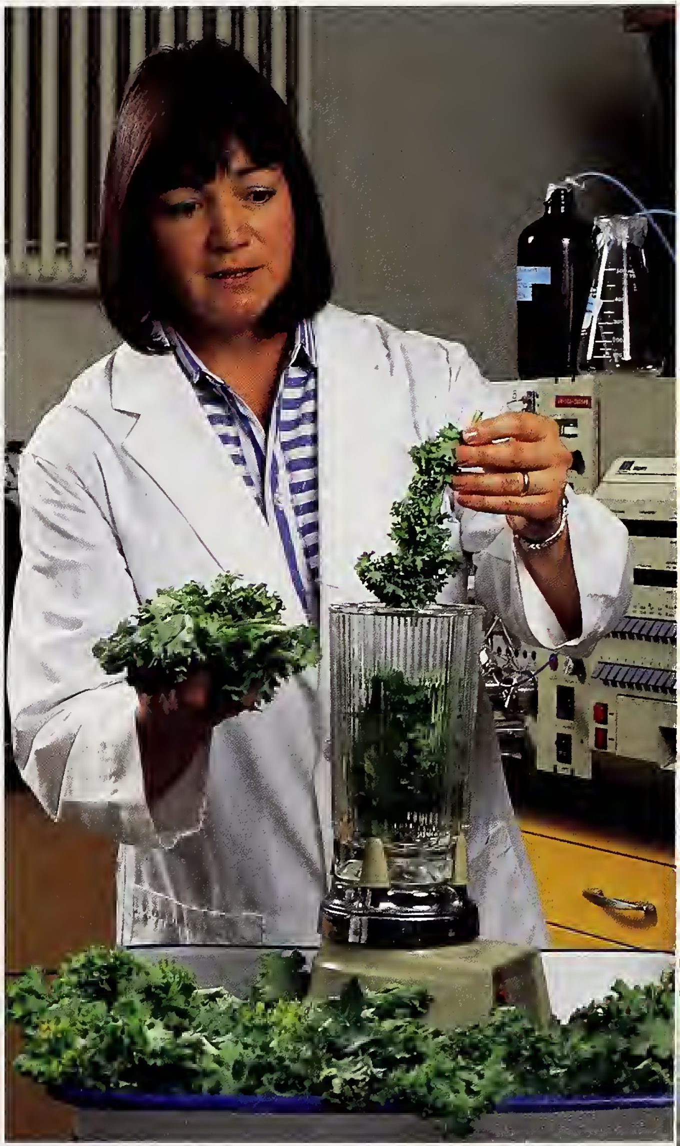
At crop fertilization levels currently used in the United States, a cup of cooked kale has an average of 102 milligrams of vitamin C and 1.8 milligrams of iron. That's about the same amount of vitamin C found in a cup of orange juice made from concentrate, but the orange juice has less iron. Collard greens have more vitamin C than kale, but less iron. Spinach has about half the amount of vitamin C seen in kale.

Reports of decreasing vitamin C with increased use of nitrogen surfaced in the 1940's with studies on grapefruit. But the availability of cheap nitrogen fertilizer in the 1950's suppressed concerns about quality in favor of yields.

Hornick says she has seen renewed interest in crop quality in the 1980's as farmers search for ways to cut chemical use, both to save money and to prevent possible pollution of the groundwater.

But Hornick sees the biggest application of her work in Third World countries, where diets are not as varied as in industrialized countries.

"They eat mostly vegetables from their own gardens and foods produced locally, with few imports," she says. "It's critical that they have the highest vitamin and mineral content possible in their fruits and vegetables because they can't make up the deficit in any other way."



KEITH WELLER

Soil scientist Sharon Hornick prepares kale grown with various rates of inorganic fertilizers for tests to determine vitamin C content. (K3208-1)

Hornick is studying the effects of fertilization on beta carotene in new carrot varieties with higher than usual beta carotene content. The human body uses beta carotene to produce Vitamin A.—By **Don Comis**, ARS.

Sharon Hornick is at the USDA-ARS Soil-Microbial Systems Laboratory, Bldg. 318, BARC-E, Beltsville, MD 20705 (301) 344-3327. ◆

Tiny Parasite Taking on Grasshoppe

Grasshoppers are among nature's most destructive insect creations, sometimes building to ravaging hordes numbering in the billions.

"No accurate count has ever been taken, but certainly thousands of people have starved to death because these insects destroyed their only food supplies," says John E. Henry, an entomologist with USDA's Agricultural Research Service. "Grasshoppers certainly are contributing to the food problems in west Africa as well as east Africa, where starvation is rampant."

Nor are grasshoppers only a Third World problem. Henry says the pests cost U.S. ranchers, farmers, government agencies, and homeowners close to $400 million annually in lost crops and grass.

As their name suggests, grasshoppers eat grass, but they'll eat nearly any kind of green plant. After crops and grasses are gone, grasshoppers will eat the bark and leaves of deciduous trees.

MICHAEL LICHTER

Entomologist John Henry and Montana State University technician Victoria Golding infect a grasshopper with a naturally occurring virus in control agent production studies. This summer, viruses collected from thousands of laboratory-infected grasshoppers will be mixed with *Nosema* and tested on 1,000 acres of western rangeland. (K3173-9)

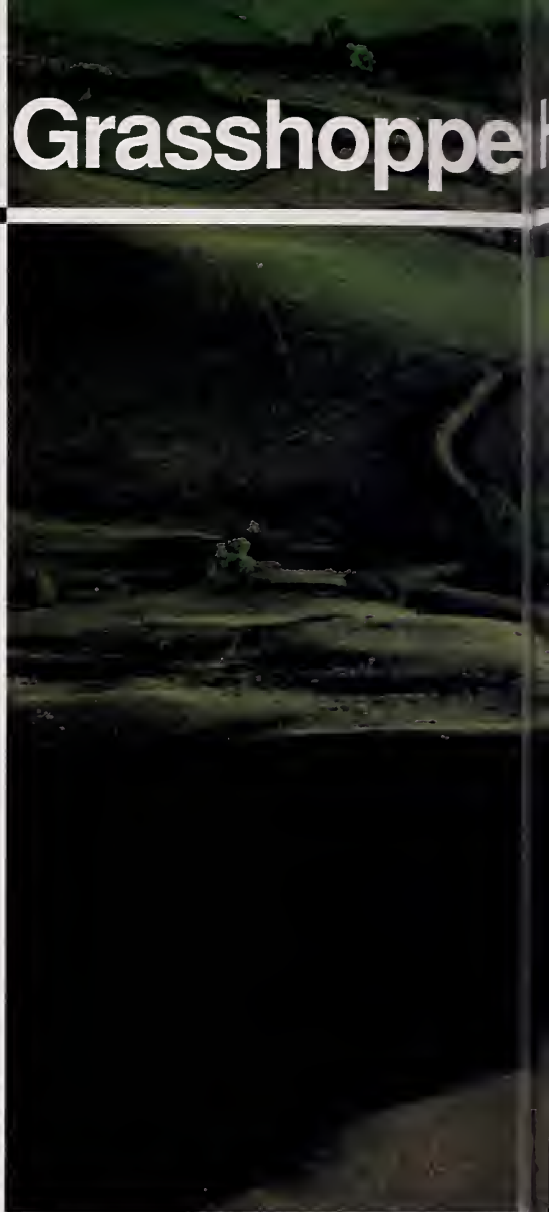
The various species of grasshoppers share a similar cycle of development. Some species lay up to 200 eggs in late summer in elongated masses in the soil. The eggs hatch in the spring and the young seek food. As the young grasshoppers grow and food becomes scarce, they migrate to new areas.

After molting five or six times during the next 40 to 60 days, they become adults and continue eating until cold weather kills them. The adults can fly to new feeding areas if local food sources are depleted.

Aerial applications of insecticides have been the primary method of controlling grasshoppers. But increasing costs of aircraft use and chemicals, along with unanswered questions about the chemicals' impact on the environment, have prompted researchers to look more closely at natural controls.

Perhaps ironically, the best natural control of grasshoppers is one that scientists cannot control: the weather. Henry says few grasshoppers would survive a cycle that might start with warm spring weather to prompt premature hatching, followed by cool temperatures that retard growth, and a short period of hot weather late in the spring to ensure complete hatching of any remaining eggs.

The pests' doom would be sealed by long periods of cloudy wet weather favorable to grasshopper diseases, a cool summer, and then an early fall to



LOWELL GEORGIA

Cropdusters release *Nosema* in a test of the biologi

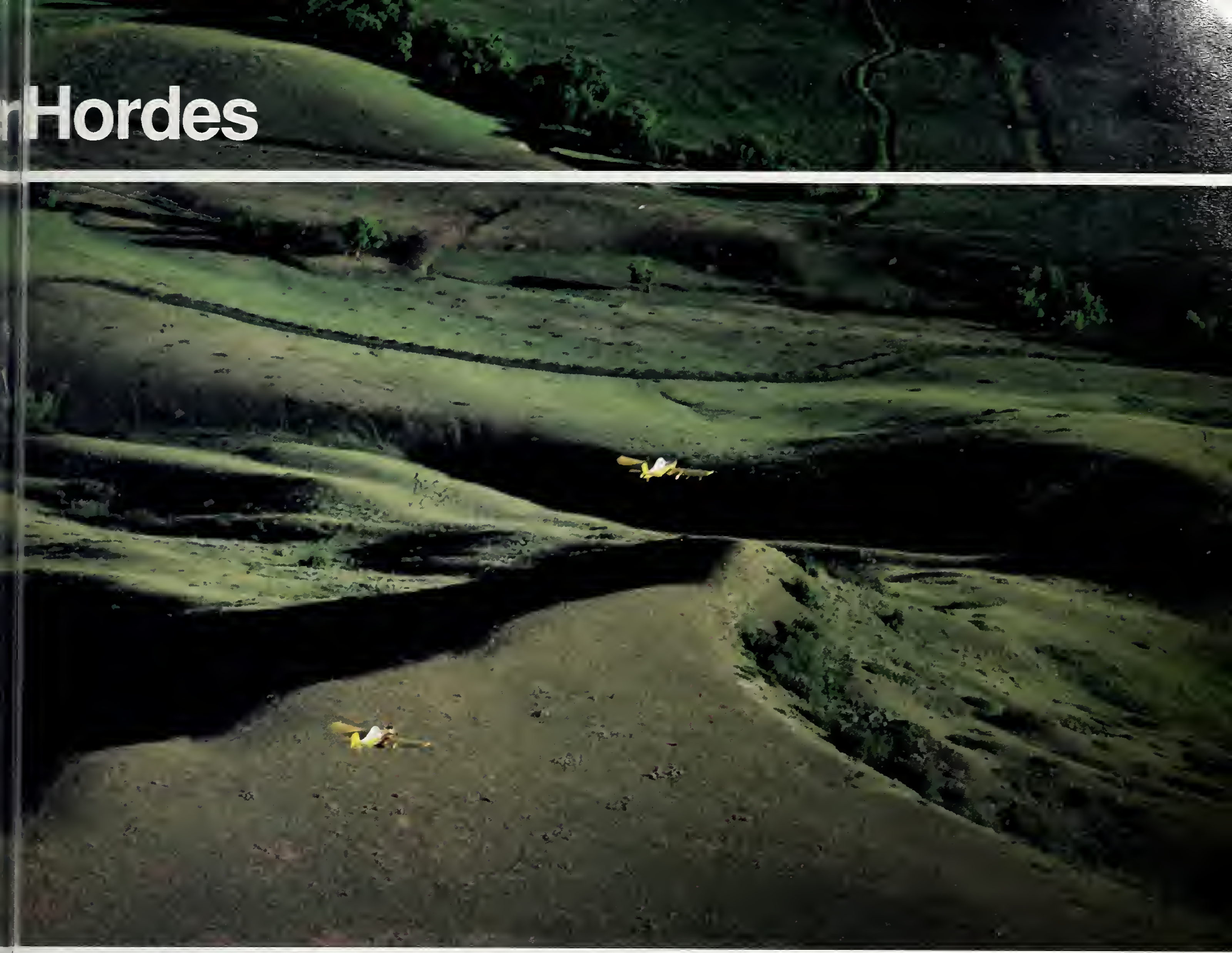
delay maturity and shorten the insects' time to lay eggs, Henry says.

But Henry and his colleagues in ARS' Range Insect Control Research Unit in Bozeman, Montana, aren't just relying on the weather to combat grasshoppers. Instead, they have succeeded in manipulating naturally occurring pathogens of grasshoppers to join the battle for control on a bigger scale.

Like most insects, grasshoppers are susceptible to infection by bacteria, viruses, fungi, and protozoa--single-celled organisms.

Bacteria and fungi can cause dramatic plagues that practically eliminate grasshoppers in specific areas. But this only occurs when a particular temperature and high humidity are ideal for rapid

Hordes



ntrol agent on grasshopper-infested Montana rangeland. (Photo K1068-4)

growth of the pathogens. Such high humidity is rare in the semiarid regions of the world where grasshoppers inflict the greatest damage.

Henry has been studying protozoa and viruses since 1961 because these two groups of pathogens are less dependent on climatic conditions.

Those studies have paid off in the form of the first protozoa to be registered for use against an insect. Now commercially available to control grasshoppers, this microscopic parasite is called *Nosema locustae*, which roughly translates as "grasshopper sickness." Sick females pass it to their offspring, and survivors cannibalize dead relatives.

Found in grasshoppers native to the United States, this naturally occurring pathogen can kill up to 90 percent of the grasshoppers in an area. The parasite eats its host's fat and multiplies until the grasshopper becomes puffy, turns white, and dies.

Henry has successfully infected 58 grasshopper species, the Mormon cricket, and a pygmy locust with *Nosema locustae* in laboratory studies.

He also has conducted rangeland studies that prove *Nosema* infects the five most numerous and destructive grasshopper species, as well as most of the 30 to 50 minor species that may be present during an outbreak.

ARS studies show that *Nosema* kills 50 to 60 percent of the grasshoppers in 3 to 4 weeks. But Henry has proved that the parasite does not infect beneficial insect species or interfere with other natural enemies of the grasshopper.

He also has devised ways to efficiently produce and store large quantities of *Nosema* in their spore stage for use during future outbreaks.

Two companies have adapted this technology for producing and storing *Nosema*. The companies are Evans BioControl Inc. of Broomfield, Colorado, and Bozeman Bio Tech in Bozeman, Montana.

As with many microbial agents, *Nosema* must be eaten by the grasshopper. Henry developed a bait using low-cost wheat bran that grasshoppers actually seek.

Henry and ARS entomologist Jerome A. Onsager have measured the persistence of *Nosema* during a grasshopper outbreak and found it active for two consecutive seasons. After that, the grass-

Entomologist John Henry (left) and research leader Jerry Onsager count grasshoppers within one of 40 metal rings periodically left overnight on rangeland. The number of grasshoppers counted provides a reliable estimate of insect population levels. (K3163-13)

An adult *Melanoplus femurrubrum* (25mm), one of more than 600 grasshopper species found in the United States. (K1066-2)

hopper populations were too low to support further spread of the organism.

"We can use *Nosema* with chemical insecticides in situations that require immediate grasshopper reductions," Onsager says. "The insecticide kills some grasshoppers within 2 days, while the *Nosema* multiplies and persists for control throughout the season."

Henry has cooperated with USDA's Animal and Plant Health Inspection Service and various State officials to demonstrate how efficiently and economically *Nosema* can control grasshoppers on the United States' Great Plains.

In other cooperative work with international organizations including the U.S. Agency for International Development and the Food and Agriculture Organization of the United Nations, Henry has helped control grasshoppers and related insects in west Africa, Argentina, China, and Indonesia. Not content with developing just one assault weapon against the grasshopper army, Henry began examining viruses as potential control agents.

"Viruses can kill within several days," he notes. "That makes them excellent candidates for control when faster action is needed.

"Coupled with *Nosema* applications, control would be both immediate and longlasting."

So far, he and colleagues have identified seven naturallyoccurring viruses in the United States and Africa that will attack grasshoppers.

Researchers are developing a DNA probe that will reduce to 5 days the time needed to show if Henry's newly identified strains or other natural controls have infected grasshoppers. Other techniques for measuring effectiveness take up to 4 weeks and are not as reliable.

Evans BioControl Inc. is conducting field tests on an entomopoxvirus for grasshopper control.

Henry originally discovered the pathogen in grasshoppers from Montana in 1965. In laboratory tests, the virus killed nearly a third of the grasshopper population.

Entomologist William P. Kemp is now developing ways to pinpoint the peaks of all five grasshopper stages based on weather records provided by weather stations throughout the western United States.

For more effective control on a larger scale, scientists are working to refine State grasshopper hazard maps that highlight the most serious infestation.

Current maps may indicate an entire three- or four-county area needs urgent control measures.

However, by applying statistical analysis to standard mapping methods, Kemp is developing State maps that pinpoint areas as small as 1,000 acres that need immediate attention.—By **Dennis Senft**, ARS.

Entomologists John E. Henry, Jerome A. Onsager, William P. Kemp and Douglas A.Streett and ecologist James S. Berry are at the USDA-ARS Range Insect Control Research Unit, S. 11th Avenue, Montana State University, Bozeman, MT 59717 (406) 994-3344. ◆

Computerized "Crystal Ball" for Wheat Growth

IMAGINE that your personal computer could tell you when to expect the first leaf on the rose bush you planted last month—or better yet, when to water, add a little fertilizer to the soil, or expect to see some flowers.

Substitute "wheat" for "rose bush," and you've just imagined PLANTEMP.

PLANTEMP is the brainchild of Elizabeth L. Klepper, an Agricultural Research Service plant physiologist, and ARS soil scientist Ronald W. Rickman. They work at ARS' Columbia Plateau Conservation Research Center at Pendleton, Oregon.

PLANTEMP is a computer modeling program that enables its users to predict when the major parts of the plant—its roots, stems, and leaves—will appear, using just weather data.

"The program can be used anywhere winter wheat is grown," Klepper says.

To zero in on particular stages of plant growth and predict how quickly their wheat will grow, farmers type daily maximum and minimum air temperatures into PLANTEMP.

In addition, the program helps growers adjust planting and irrigation methods and schedules for more vigorous early plant growth.

Klepper's work with wheat plants means greater profits for agricultural producers and a better understanding for scientists of how this vital plant manufactures the nutrients needed by both people and livestock.

To develop PLANTEMP, Klepper and co-workers diagramed the order in which individual wheat leaves, shoots, and roots usually appear on the plant.

"With too much stress, such as drought or too little fertilizer, roots may be shorter than usual and leaves may be omitted or produced later than shown on our diagram," says Klepper.

By comparing this wheat-development sequence with the way their own crops are growing, grain producers can "read" the stress history of a plant and, if possible, improve the following year's crop.

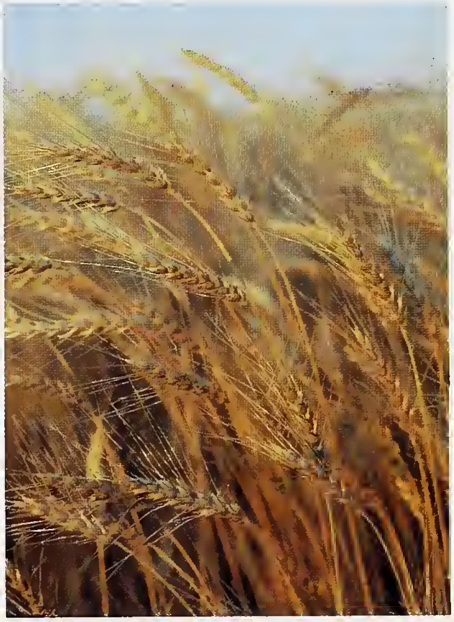
"It's almost like reading the rings of an ancient tree to find out when it burned, was attacked by insects, or suffered a drought."

"It's almost like reading the rings of an ancient tree to find out when it burned, was attacked by insects, or suffered a drought," says Klepper.

According to Klepper, many farming decisions—such as when to apply a herbicide or fertilizer or when to irrigate—require knowing the stage of development of the crop. Poorly timed applications can damage the crop or reduce the effectiveness of weed, insect, or disease controls.

A publication describing the PLANTEMP software is available from Oregon State University (OSU) Extension Service, Corvallis, Oregon. Request publication number EM 8308, "PLANTEMP Version 2.0."

"Although PLANTEMP was originally designed to predict the growth stages of wheat, the Soil Conservation Service has modified the software to conserve highly erodible farmland in the Pacific Northwest," notes Hans Krauss, an SCS agronomist in Spokane, Washington.


©GRANT HEILMAN, INC.

Wheat production practices can be fine-tuned using the PLANTEMP computer modeling program.

"By modifying PLANTEMP and incorporating it into the cropping management factor of the Universal Soil Loss Equation," Krauss says, "SCS can accurately predict the amount of vegetative cover produced by fall-seeded winter wheat.

"These predictions provide residue and seeding-date alternatives for producers seeking a farming system that will protect their soil."

Now Klepper and Rickman are studying and mapping the development of spikelets, which contain the wheat grains. Knowing when the different parts of the wheat head form will provide valuable information for managing crops. For example, nutritional stress, such as too little nitrogen, can reduce the number of spikelets that form. Other environmental factors, such as high temperatures, may abort some kernels.

"Before we can manage crops for more productive grain heads, we need to understand their growth history," Klepper says.—By **Howard Sherman,** ARS.

Elizabeth Klepper is at the USDA-ARS Columbia-Plateau Conservation Research Center, P.O. Box 370, Pendleton, OR 97801 (503) 276-3811. ◆

Uncovering Food Pathogens' Nasty Secrets

JAMES L. Smith isn't a modern-day Sam Spade, but he is hot on the trail of missing millions—millions of bacteria, that is.

Smith is a microbiologist at ARS' Eastern Regional Research Center at Philadelphia, Pennsylvania. He works in the center's Microbial Food Safety Unit, trying to ensure that food processors get an accurate reading on their products when they check for potentially harmful bacteria.

The problem is the testing material, called bacterial growth medium. The medium is designed to be selective—in other words, to allow the bacteria in question to show up while others are inhibited. But that selectivity sometimes goes too far, says Smith.

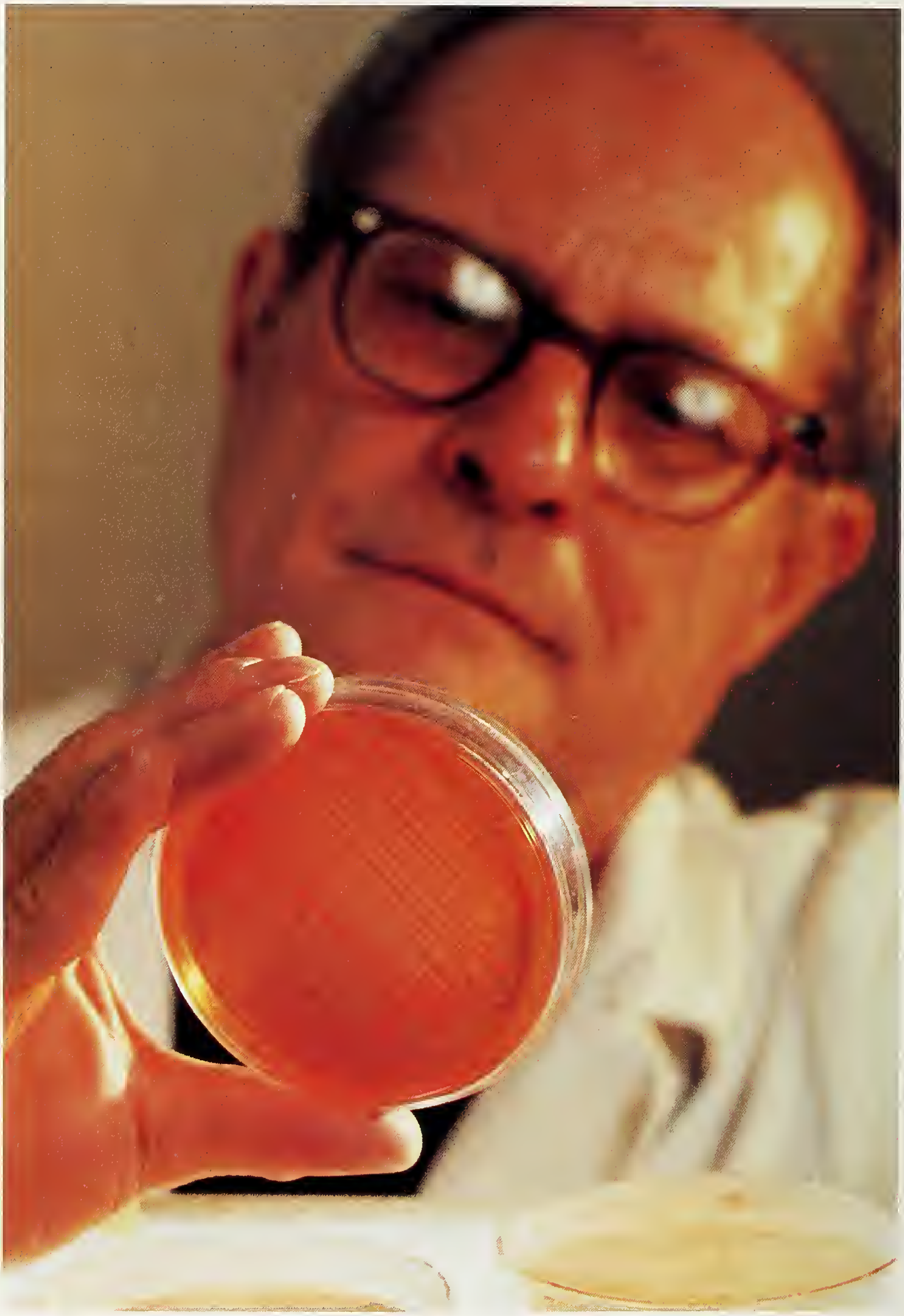
"If the food has been underprocessed, the bacteria may be injured rather than killed," Smith explains. "The selectivity that certain additives, such as antibiotics or salts, can give to a bacterial medium to inhibit undesired bacteria usually prevents the growth of injured cells of the type of bacteria you want to count.

"Since injured cells can grow and repair under the right conditions, the use of selective media to determine bacterial numbers in underprocessed foods usually leads to a lower count of potentially harmful bacteria."

Unfortunately, if injured bacteria in a food product do repair, then they will pose a threat to consumers—a threat unsuspected by processors who get inaccurately low bacterial counts, Smith notes.

"Imagine you're a food microbiologist working in a plant, and they tell you there's a limit of no more than a million bacteria allowed per gram of your food," he says. "So you check your food sample on a medium that has an agent that won't let the injured cells survive, and you only find a million bacteria per gram, so the food passes the test.

"But if you were to test the sample on some other medium that doesn't have the selective agent, you might find a billion bacteria and the food would be unacceptable."



KEITH WELLER

Microbiologist James Smith checks growth medium for visual evidence of harmful bacteria. (K3206-20)

Smith, who is hearing-impaired, isn't just pulling those numbers out of some scientific hat. In tests involving the food pathogen *Listeria monocytogenes*, he heated a billion bacteria per milliliter at 52 degrees Celsius for 60 minutes to injure the cells. He then counted those bacteria on a medium that contained sodium chloride, which will not let the injured cells grow. The

count indicated only a million bacteria were present.

But when he tried the same techniques on a medium that did not contain sodium chloride, the truth came out: There were approximately a billion *Listeria* swarming around, 1,000 times the number previously indicated.

Listeria is of concern because the food pathogen can grow in cold temperatures, rendering refrigeration less effective as a defense.

However, it generally is not lifethreatening for those it infects unless they are pregnant or have a compromised immune system, such as people with acquired immune deficiency syndrome, or AIDS. In those cases, a *Listeria* infection can be fatal, says Smith.

That there is any sort of medium at all on which to check for *Listeria monocytogenes* is a credit to the Philadelphia lab.

KEITH WELLER

Fluorescent light illuminating colonies of bacteria in growth medium aids researchers in making counts of organisms present to help ensure food safety. (K3207-13)

The medium used, modified Vogel-Johnson agar, was developed last year by Robert L. Buchanan, research leader of the Microbial Food Safety Unit.

"That medium eliminates most of the other organisms present in food," Smith says.

"But it also prevents the injured *Listeria* from repairing, so you only get a count of the noninjured cells.

"We're looking for something that we can add that will give the count of both injured and noninjured cells.

"If we could add something to modified Vogel-Johnson that will let us count the injured cells, too, we could get a more accurate count of bacteria levels. We've been working on this for a couple of years and feel that we have some good leads."

Along the same lines, Smith is looking for media that will allow microbiologists to count injured *Shigella* species using *Shigella flexneri* as a model. *Shigella flexneri* causes severe diarrhea. The pathogen can be found in both food and water and is the second largest killer of children in Third World countries.

"I've tested about 25 media," Smith reports. "Some were better than others; the addition of sodium pyruvate to some of the selected media permitted the growth of injured *Shigella*."

Smith also is studying *Shigella flexneri's* virulence plasmid. The plasmid carries the genes that enable the bacteria to invade a victim's intestinal tracts.

The same plasmid also bears a gene that allows the microorganism to take up a red dye called congo red. Growth of virulent *Shigella flexneri* on an agar medium with congo red results in red bacterial colonies containing cells capable of intestinal invasion.

"We've looked at the effect of various food additives—salts, sugars, etc.—as well as variations in pH and temperature, and we find there's no loss of the plasmid," Smith says. "Growing *Shigella* in the presence of certain selective agents in bacterial media can result in the loss of the virulence plasmid. But the acidity level of food or the temperature at which the food is processed don't do it."—By **Sandy Miller Hays**, ARS.

James L. Smith is in Microbial Food Safety Research, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118 (215) 233-6520. ◆

Pinning Down Copper's Place in Diet

When it comes to cookware, electrical wire, and even the 2 cents in your pocket, copper's importance is easily recognized.

The mineral also is essential to the body. But science isn't sure how much copper is needed in the human diet. For that reason, you won't find the recommended daily allowances for copper listed on the side of your breakfast cereal box along with those for iron, zinc, and magnesium.

Phyllis E. Johnson, a chemist with the Agricultural Research Service, is tackling at least one part of the mystery by studying how copper is absorbed by the human body from various foods. She has been studying copper and its role in human nutrition since 1982.

Copper may play a significant role in maintaining healthy cholesterol and blood-sugar levels and keeping the body's blood pressure in line, according to Johnson.

It also may help prevent the body's connective tissues from breaking down. A stronger circulatory system lessens the chance of heart disease.

Studies of copper in laboratory animals have proven that the mineral works to strengthen connective tissue, Johnson says.

The body's absorption of other minerals from foods has been well documented. For instance, Johnson says, the body absorbs approximately 5 percent of iron contained in foods, while absorption of zinc and other minerals is much higher.

"The body works very hard to maintain a status quo," she says. "We know that if our diet is low in copper, the body tends to absorb more."

Copper absorption studies have been conducted on animals and human volunteers at ARS' Human Nutrition Research Center in Grand Forks, North Dakota. Participants ranged from 21 to 80 years old.

The researchers injected a nonradioactive, naturally occurring form of copper into foods known to be high in copper content, such as goose meat, peanuts, and wheat. The nonradioactive copper was sprayed on the wheat because of the difficulty in injecting the material into every kernel of grain.

BRUCE FRITZ

As part of studies on human dietary requirements for copper, ARS chemist Phyllis E. Johnson, at Grand Forks, North Dakota, measures the amount of copper absorbed from foods eaten by volunteers. (K1794-2)

Volunteers in the program were housed at the ARS lab and ate only the copper-treated food, allowing scientists to keep tabs on copper absorption by the body.

Volunteers' food intake was closely watched. For example, whenever a volunteer left the center, he was accompanied by a chaperon, Johnson says. She says it is important that no food or beverage outside the controlled diet enter the body.

The volunteers were required to eat three daily meals of the high-copper foods used in the study. By controlling the volunteers' diet and eating habits, scientists were able to discover a consistent copper absorption pattern.

When studying copper absorption in animals, researchers were able to study organ samples and use radioactivity. But the human study was restricted to analyzing feces and body secretions.

Other minerals and vitamin C have an impact on the amount of copper absorbed, so researchers also controlled the levels of vitamins and other minerals in the volunteers' diet, according to Johnson.

The study showed that copper absorption by the volunteers ranged from about 50 percent to 70 percent in adult men and women.

The study also indicated a difference in copper absorption from foods consumed as liquids or as solid food. Johnson says the body tends to absorb more copper from solid foods than from liquid foods with comparable levels of copper.

Researchers also studied copper absorption in infants from both formula and breast milk. Johnson says infants tend to absorb 80 percent of the mineral, with little difference between formula and breast feeding.

Too much copper in the diet can be toxic, but that is unlikely to happen to people eating normal diets, she says.

"People who take copper supplements in excessive amounts could get sick," Johnson says. "The real issue is, what is the minimum requirement?"

Johnson now is studying ways the body controls copper absorption.—By **Bruce Kinzel**, ARS.

Phyllis Johnson is at the USDA-ARS Human Nutrition Research Center, P.O. Box 7166 University Station, Grand Forks, ND 58202 (701) 795-8456. ◆

Deadliest Days of Drought Stress

Microscopic reconnaissance inside seeds is turning up information that may help farmers who raise crops to produce seed for next year's planting.

Using computer analysis of photography of developing cotton seeds, plant physiologist Eugene L. Vigil has discovered a crucial 10-day "window" of drought stress. Vigil found that when cotton plants are hit with drought 30 to 40 days after flowering, they produce seed that sprouts only 20 percent of the time. Seed from plants drought-stressed after that 10-day period sprout 100 percent for the next crop.

The computer technology used by Vigil and technician Chris Pooley assigns colors to different shades of gray in photographs. The technology was developed for use with air- and space-reconnaissance photographs.

"But it is ideal for freeze-framing biological events, such as seed ripening," says Vigil, who works in the ARS Climate Stress Laboratory at Beltsville, Maryland.

Vigil analyzes electron microscope photographs to find ways to improve the quality and amount of oil and protein in oilseed crops such as cotton, soybeans, and sunflowers.

A computer program enables him to identify areas of protein storage and paint the protein with a chosen color. The computer program paints green the surrounding tissue that fails to fill up with protein.

The color analysis shows clearly that ripening seeds that were drought-stressed during the crucial 10-day period had mostly green-colored areas, meaning the tissue had almost no protein. Seeds hit with drought after the 10-day period had storage areas filled with protein, which appeared red in the computer images.

Vigil describes seeds as the "food sinks" of plants. After leaves, stems, and roots collect food through photosynthesis and mineral absorption, the plant matures and much of the collected food fills the seeds.

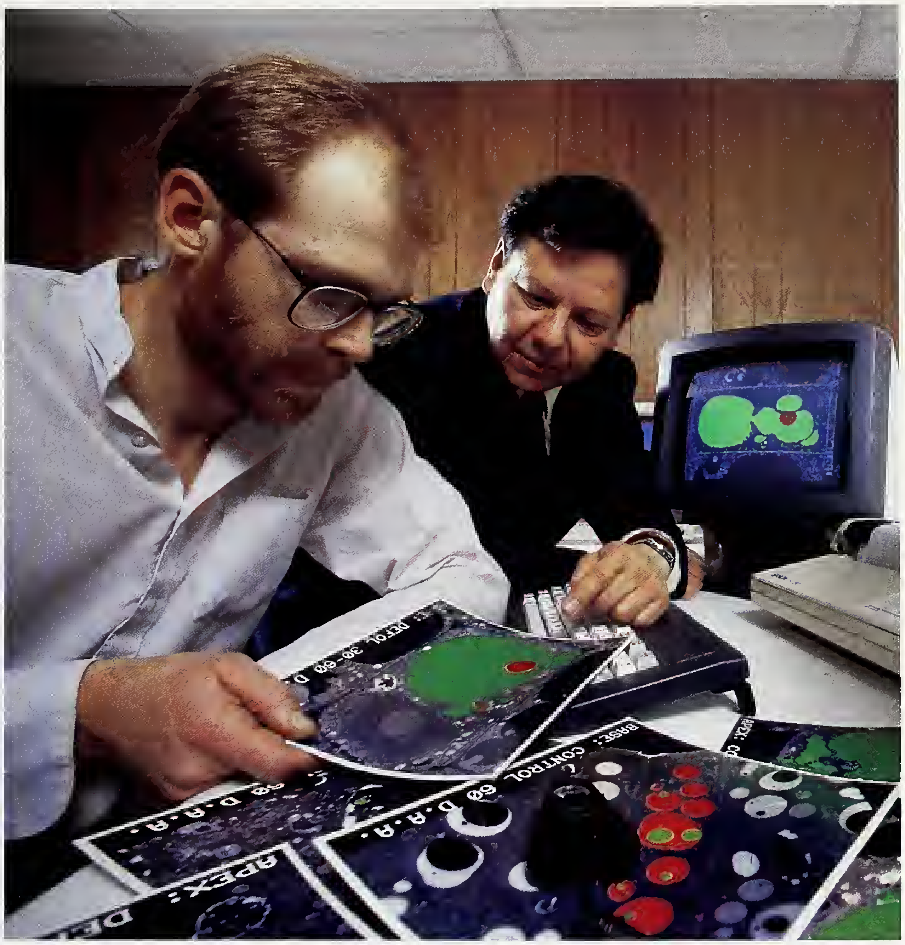
In his experiments, Vigil simulated severe drought by stripping leaves from plants as they matured. He then randomly selected seeds from more than 50,000 cottonseed bolls from stripped and unstripped plants.

"I was surprised at the sudden formation and onset of protein filling between 30 and 40 days after flowering," says Vigil. "I had expected a more gradual process of ripening."

The 10-day window of drought stress also may affect other crops such as corn, Vigil says.

He says the work could someday lead to a simple test kit for seed quality.—By **Stephen Berberich**, ARS.

Eugene L. Vigil is in the USDA-ARS Climate Stress Laboratory, Beltsville, MD 20705 (301) 344-1649. ◆



KEITH WELLER

Biological technician Chris Pooley and plant physiologist Eugene Vigil examine electron micrographs of cotton root cells to determine areas for image analysis. (K3203-1)

Moth Recruited to Gnaw Knapweed

As weeds spread across millions of acres of Northern Plains rangeland, crowding out more desirable forage grasses, scientists are aggressively fighting back by releasing insects that devour the noxious invaders.

"Biological control will not solve ranchers' problems overnight," says Sara S. Rosenthal, an entomologist with the Agricultural Research Service. "However, such biological control is relatively inexpensive, longlasting, and ecologically sound."

Among the weeds, four kinds of knapweed have expanded their territory greatly in recent years: spotted knapweed, diffuse knapweed, Russian knapweed, and yellow starthistle.

Rosenthal says many insects are needed in the biological control arsenal to combat spotted knapweed, which now infests more than 2.5 million acres in Montana alone.

One of the most promising weapons already in hand is a bright yellow, inch-long moth called *Agapeta zoegana*—the yellow-winged root moth.

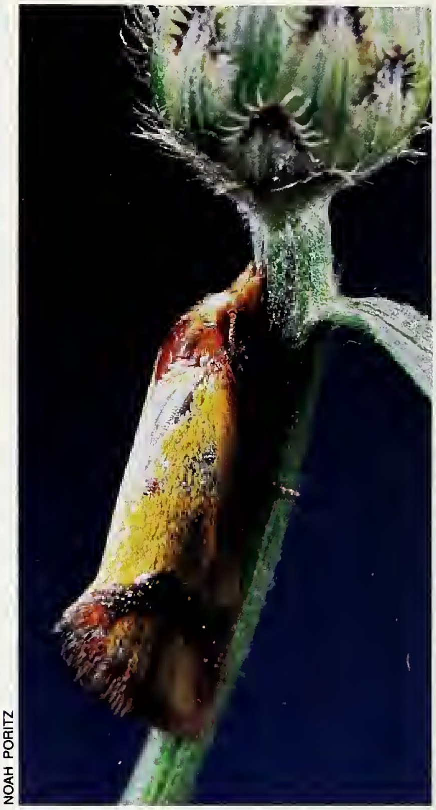
The yellow-winged root moth has an international background of sorts. It was originally studied in Switzerland to ensure that its only diet was knapweeds and not other food or fiber plants.

Then its eggs were released in Canada in 1982 and in Montana in 1984, where populations have gradually become established. Rosenthal released both eggs and larvae in 1987 at four locations in Washington state, and the moth rapidly established itself there as well.

The yellow-winged root moth joins two other root moths, a root boring beetle, two seed head flies, and one seed head moth already introduced into knapweed-infested areas.

MICHAEL LICHTER

Entomologist Sara S. Rosenthal inspects early spring knapweed near Bozeman, Montana. (K3179-13)



NOAH PORITZ

Adult yellow-winged root moth (*Agapeta zoegana*, 22mm in length) on knapweed.

NOAH PORITZ

Cut-away knapweed root section attests to the voracious appetite of *A. zoegana*.

"The yellow-winged root moth lays its eggs near the base of spotted knapweed plants," explains Rosenthal. "After the eggs hatch, the emerging larvae bore into the weeds' roots. This destruction interferes with nutrient movement in the weed, sapping it of its strength."

In addition, disease-causing microorganisms are able to enter the weed's roots through the holes made by the emerging larvae.

Rosenthal, who works at ARS' Biological Control of Weeds Research Unit at Bozeman, Montana, says it could take several years before the moth population grows enough to make a real impact on spotted knapweed.

She says the insects were collected in Austria and Hungary, where spotted knapweed is a native plant, but is not considered a weed.

"In Eurasia there are many kinds of insects attacking all parts of the knapweed plant, including the flowerheads, seeds, stems, leaves, and roots," she says.

"Knapweeds are not a problem there because they grow as scattered individuals instead of in the thick populations found in North America. We are trying to import a variety of insects and plant diseases to obtain a similar balance here," says Rosenthal.—By **Dennis Senft**, ARS.

Sara S. Rosenthal is at USDA-ARS' Biological Control of Weeds Research Unit, Rangeland Insect Laboratory, Montana State University, Bozeman, MT 59717 (406) 994-4892. ◆

Seeds Doze Safely Under Laughing Gas

Sharon Sowa's search for newer and better ways to preserve seeds has turned up some unusual tools—such as laughing gas.

Sowa, an Agricultural Research Service chemist, is encouraged by the early results of her nitrous oxide experiments and will be investigating other gases to find ones best suited for storing seeds.

"It's insurance against their extinction and keeps seeds available to breed agricultural plants for drought tolerance, disease resistance, and other traits," says Sowa.

Using nitrous oxide, Sowa slowed respiration in the cells of snap beans by 35 percent, enough to anesthetize the seeds. Air that's 80 percent nitrous oxide—a component in human anesthetic—was blown over the seeds. Thirty minutes later she began reviving them from their nap.

Like Rip Van Winkle, Washington Irving's ne'er-do-well character who slept for 20 years in the Catskill Mountains of New York, all the seeds eventually woke up and germinated normally. Like humans, the seeds recover quickly when brought into fresh air.

Now Sowa is trying the anesthetic on hard-to-store seeds so they can be kept safely.

Sowa, at ARS' National Seed Storage Laboratory in Fort Collins, Colorado, spelled out her goal: "to treat seeds someday much like doctors treat their patients in surgery. Anesthetics are used to slow human respiration during surgery. They also slow the life processes inside seeds."

Most seeds can be stored safely either in temperature-controlled rooms or frozen in liquid nitrogen. But that's not the case for seeds that come mainly from tropical and subtropical plants such as passion fruit and macadamia.

"These seeds are usually large and contain up to 50 percent moisture compared with about 10 percent for most vegetable seeds," Sowa explains. "They can't be dried, so their high moisture content results in a short storage life."

Sowa says nitrous oxide is what scientists call a "permeable molecule"—it goes in and out of plant cells very easily, without causing any damage. Exactly how it works to slow respiration in humans and seeds is unknown.

"We also are looking for ways to store citrus and other tree crops," she says. "Currently, we must keep trees growing in orchards if we want to preserve their unique genetic traits for future breeding experiments.

"If just a few cells or buds from these trees could be anesthetized, then regrown into trees when a tree breeder needed them, we would have a much cheaper and safer way to store them."

The National Seed Storage Laboratory's mission is to keep and preserve the 240,000 accessions now in cold storage at Fort Collins. Limited numbers of seeds are sent out on request to breeders and scientists in the United States and throughout the world.—By **Dennis Senft**, ARS.

Sharon Sowa is in USDA-ARS Plant Germplasm Preservation Research, Fort Collins, CO 80523 (303) 484-0402. ◆

JACK DYKINGA

Chemist Sharon Sowa prepares subcellular bean seedling extract for respiration measurement as part of her studies on seed deterioration and genetic changes during storage. (K3049-4)

Solving Plant Hormone Puzzle

Just like people, plants are sometimes at the mercy of their hormones.

One of the five most powerful hormones in any green plant is abscisic acid, or ABA. Scientists are fascinated by ABA because it apparently plays a major role in many key events of a plant's life. Once ABA's secrets are unlocked, science might be able to give growers more control over the plants they raise.

In wheat, abscisic acid keeps seeds from sprouting too soon and helps plants cope with drought, says M.K. Walker-Simmons, a plant physiologist with the Agricultural Research Service at Pullman, Washington.

Premature sprouting is bad news for everyone, from farmers to exporters, because sprouted kernels lower the value of properly ripened grain used to make flour.

"We're trying to determine how ABA affects the genes of dormant seeds—seeds that haven't germinated yet," says Walker-Simmons.

"ABA might cue genes to produce a distinctive protein or nucleic acid that would be a reliable indicator of dormancy. Such a marker would help genetic engineers and plant breeders develop wheat that sprouts when it's supposed to."

Researchers know little about how ABA works or what enzymes produce it. "We know the amount of ABA increases dramatically in leaves when drought hits wheat and barley plants," says Walker-Simmons.

She measures ABA levels with her own test, capable of accurately detecting tiny amounts of ABA in a single grain of wheat or barley.

With this test, Walker-Simmons and Washington State University plant physiologist Robert Warner recently identified a mutant barley plant that doesn't produce the hormone in response to drought. By comparing the mutant with normal barley plants, the researchers hope to identify enzymes the mutant lacks.

Those missing enyzmes might be the unknown ones plants require for manufacturing ABA. Their identity will be an important new clue for genetic engineers who want to adjust ABA production so that tomorrow's crops can better withstand drought.

A similar test, developed by ARS researchers Shirley M. Norman, Stephen M. Poling, and Vincent P. Maier (now retired) at Pasadena, California, has exposed some of the workings of ABA in citrus.

Like Walker-Simmons, Norman has seen ABA levels soar in plants that don't get enough water. Norman's experiments with lemon plants show that thirsty plants can have 60 times more ABA than when they have been well watered.

In her search for answers on how plants make ABA, Norman is applying chemicals known as growth inhibitors to lemon seedlings. Her earlier work with another organism—a mold that has an unusually high amount of ABA—showed that growth regulators hold back ABA production.

"If the same happens in citrus, we may be able to discover how a normal plant—one that isn't treated with a growth regulator—would go about making ABA," she says.

Once the riddle of ABA production is solved, scientists could intervene in the natural aging of crops. Norman looks forward to the day when ABA levels in tons of warehoused lemons or oranges could be safely and gently altered, giving consumers a fresher tasting harvest.—By **Howard Sherman and Marcia Wood**, ARS.

M.K. Walker-Simmons is at the USDA-ARS Wheat Genetics, Quality, Physiology, and Disease Research Unit, Johnson Hall, Room 209, Washington State University, Pullman, WA 99164-6420 (509) 335-8696. Shirley M. Norman and Stephen M. Poling are at the USDA-ARS Fruit and Vegetable Chemistry Laboratory, 263 South Chester Avenue, Pasadena, CA 91106 (818) 796-0239. ◆

Cooking Creates Host of Cholesterol Compounds

When the subject is cholesterol, all too often what you see isn't quite what you get.

Cholesterol, pegged as a villain in human heart disease, is a chemical chameleon. When cholesterol meets air at high temperatures, such as during frying or some forms of processing, small portions change into compounds called cholesterol oxides. When this happens, food processors find themselves playing in a whole new ballgame.

As many as 80 different cholesterol oxides are known to exist, although only 10 to 15 are commonly seen. Two of the many cholesterol oxides that might appear, the alpha-epoxide and the beta-epoxide, have been reported to be carcinogenic in experiments with animals.

But predicting which cholesterol oxides might pop up in a food product isn't as much of a guessing game as it once was.

That is because of information that emerged from findings in a 5-year study completed in August 1988 by scientists in the Food Safety Research unit of the Agricultural Research Service's Eastern Regional Research Center at Philadelphia, Pennsylvania.

In the study, organic chemist Gerhard Maerker put pure cholesterol in water and pumped air into the water at 80°C (176°F) to bring the cholesterol in contact with oxygen. This resulted in the formation of a variety of cholesterol oxides.

"We found out a lot about what compounds come first and what come later," says Maerker. "The hydroperoxides are the first to appear; they come and go and form other compounds, including the epoxides.

"To stop cholesterol oxidation, we have to know more about the manner in which cholesterol oxidizes.

"There are ways food processors can use the information we've obtained from this study.

"For example, a cheesemaker might be using a peroxide to kill bacteria in his cheese. But with this information in hand, he may want to use something else so he can keep the oxides out of his product."

As a sidelight to the cholesterol dispersion work, Maerker and his associates took a closer look at what is likely to happen to the reportedly carcinogenic epoxides after they're eaten by humans.

"We made up synthetic gastric juices, put them in a flask with the epoxides and took samples every few minutes," he recalls.

"We found the epoxides aren't really as fearful as we thought. They don't survive the acidity of the stomach. The gastric juices break the epoxides into an oxide called triol, which is not carcinogenic."—By **Sandy Miller Hays**, ARS.

Gerhard Maerker is in the USDA-ARS Food Safety Research Unit, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118 (215) 233-6446. ◆

More Precision in Brucellosis Tests

Brucellosis, or Bang's disease, is a major cause of abortion in cattle. Caused by the bacterium *Brucella abortus*, the disease causes estimated losses of more than $80 million annually, reduces cattle fertility, and lowers milk yields.

In humans, the disease is called "undulant fever," and may strike people who handle infected animals or fres carcasses of infected animals.

Brucellosis research has been under way at ARS' National Animal Disease Center at Ames, Iowa, since the early 1960's.

An important recent development in that work is the discovery of a new use for the western blot test, a highly specific test used to identify the AIDS virus and several other viral diseases.

Louisa B. Tabatabai, a research chemist at the center, is using the test to distinguish between cattle infected with brucellosis and those that were vaccinated as calves with *Brucella abortus* strain 19.

"It's very difficult, if not impossible, to distinguish vaccinated from infected cattle using current diagnostic tests," says Tabatabai. For more than 40 years, standard blood tests have been used for diagnosing brucellosis in livestock.

"Despite some improvements, the antigen used in most tests cannot distinguish between two types of antibodies," Tabatabai says.

In the western blot procedure, a protein isolated from strain 19 reacts with blood from infected cattle, but does not react with blood from cattle vaccinated as calves.

The attractive feature of the western blot test is that no calfhood-vaccinated animals were detected as infected with brucellosis.

But, as with otheϼ, conventional blood tests, blood from adult-vaccinated animals may pose problems even with the western blot procedure.

To date, Tabatabai has isolated seven proteins from *Brucella abortus*. She continues to study each individual protein in the hope of finding one, or a combination, that will be most effective for use in a vaccine or as a complementary diagnostic test.—By **Linda Cooke**, ARS.

Louisa Tabatabai is in Brucellosis Research, National Animal Disease Center, 2300 Dayton Avenue, Ames, IA 50010 (515) 239-8370. ♦

Fat Cells, Fat Chicks

Well, it looks like chickens are going to have to lose some fat—if agricultural scientists have their way.

In today's "slim-down" society, consumers want leaner meat. That is one reason they have been turning to chicken meat, which is relatively low in fat. Since 1970, per capita consumption of poultry has jumped about 50 percent.

Still, modern chickens are too fat, a fact that's costing the poultry industry millions of dollars annually, according to Teresa L. Blalock, an animal physiologist with the Agricultural Research Service.

Blalock and other researchers are looking for the proteins, hormones, or enzymes that control fat cell size in chickens. In their search, they have discovered, for the first time in chickens, the protein adipsin.

"Adipsin is involved in the control of fat cell size in mice," says Blalock, who works at ARS' Georgetown, Delaware, poultry research laboratory. "In our research, we will try to see if it plays the same role in chickens."

Blalock and her colleagues are working to develop specific genetic probes to determine which genes control the production of adipsin in chickens. This could lead to the control of adipsin either through biochemical manipulation or genetic engineering, and ultimately to leaner poultry.

Chicken fat is not distributed throughout the muscles. Instead, it is found under the bird's skin and in pockets such as in the large piece of stomach fat that is discarded in the kitchen or packing plant.

This waste costs farmers more than $300 million annually in chicken feed, costs passed on to the consumer who ends up paying the same amount per pound for fat as well as meat.

Reduction of chicken fat may also help the U.S. trade balance. Chicken fat turns rancid even when frozen. This limits the amount of fresh frozen chicken the United States can send overseas.

"In the last 30 years, chickens have been bred to grow faster and larger," notes Blalock. "In fact, it only takes 6 to 7 weeks to get chickens to market weight—half the time it used to take.

"Unfortunately, this rapid increase in size has been accompanied by a disproportionate increase in fat."

In other livestock, such as cows and pigs, injections of growth hormone cause the animals to grow faster and put on lean meat.

However, even though chickens produce growth hormone, similar injections do not cause either body growth or lean meat.

This may mean that a different mechanism is required in chickens for reducing fat, Blalock says. She says the detection of adipsin in chickens is an important step toward reducing fat in the bird.—By **Vince Mazzola**, ARS.

Teresa L. Blalock is in USDA-ARS Poultry Research, Georgetown, DE 19947 (302) 856-0046. ♦

U.S. Department of Agriculture
Agricultural Research Service
Rm. 318, B-005, BARC-West
Beltsville, MD 20705

Official Business
Penalty for Private Use—$300

To stop mailing ☐
To change your address ☐
Send mailing label on this magazine and your new address to above address.

Bulk Rate
Postage and Fees Paid
U.S. Department of Agriculture
Permit No. G-95

☐ **YES** Please send me 10 issues of **Agricultural Research** SN 701-006-00000-3

Charge your order.
It's easy!

1. The total cost of my order is $14.00 (International customers please add 25%.) All prices include regular domestic postage and handling and are good through Sept. 89. After this date, please call Order and Information Desk at (202) 783-3238 to verify current price.

Please Type or Print

2. ______________________
(Company or personal name)

____
(Additional address/attention line)

____
(Street address)

____
(City, State, ZIP Code)

( )______________________
(Daytime phone including area code)

3. Please choose method of payment:

☐ Check payable to the Superintendent of Documents

☐ GPO Deposit Account

☐ VISA, or MasterCard Account

______________________ *Thank you for your order!*
(Credit card expiration date)

____
(Signature)

4. Mail To: Superintendent of Documents, Government Printing Office, Washington, DC 20402-9325